Additional file 1

Supporting text for Figure 1. Pharmacology-network based drug repurposing for CMT1A disease.

(A) Three principal pathways regulating expression of *PMP22* gene through extracellular GPCR signalling in Schwann cells. The presumed crosstalk between cAMP pathway, neurosteroid-mediated signalling and mutually-balanced PI3K-AKT/ERK kinase cascades provides functional signals regulating the expression of *PMP22* gene. cAMP signalling is implicated in different aspects of Schwann cell biology and influences their differentiation and myelin formation [1, 2]. cAMP/PKA module is able to modify the activity of several transcriptional factors implicated in the transcriptional control of *PMP22* gene. In cell cultures, cAMP increases the expression of PMP22 protein acting presumably, through down-regulation of inhibitory effect of cAMP-dependent silencer element in the promoter region of *PMP22* gene [3, 4]. The physical contact of Schwann cells with neurons is thought to control the intracellular levels of cAMP, modifying Schwann cell response to growth factors [1]. Moreover, the differentiated state of Schwann cells depends on the counter-balanced activation of ERK and PI3K-AKT pathways by growth factors, mediated through receptor-tyrosine kinases, with PI3K-AKT signalling promoting differentiation and expression of myelin proteins [5, 6]. It was shown that canonical cAMP-PKA-CREB pathway synergistically and in a dose-dependent manner enhances pro-myelination effects of NRG1 signalling likely mediated by AKT kinase and EGR2 transcription factor, a positive regulator of myelination program and *PMP22* transcription in Schwann cells [7, 8].

Neurosteroid progesterone and its derivatives (DHP and allopregnanolone) play an important role in myelin formation acting as autocrine regulatory factors. Transcriptional up-regulation of *PMP22* gene by the neurosteroids can be mediated or modified by cAMP signalling; thus,

treatment with allopregnanolone, a positive allosteric modulator of $GABA_A$ receptors, increases the level of intracellular cAMP and CREB phosphorylation in Schwann cells [4, 9–11]. We expected that these convergent signalling pathways in Schwann cells can be simultaneously regulated by different G-protein coupled receptors (GPCRs) either directly or via crosstalk with receptor tyrosine kinases, as it was shown in other cellular settings [12, 13]. Accordingly, pharmacological modulation of the G-protein coupled receptors opens the possibility for developing robust and safe combinational therapeutics decreasing the deleterious excessive expression of PMP22 protein and restoring the differentiation program in CMT1A Schwann cells.

GABA(B)R: metabotropic GABA receptor; OPRs: opioid receptors; FLNA: filamin A, alpha; POMC: proopiomelanocortin; PENK: proenkephalin; PDYN: prodynorphin; CHRMs: muscarinic receptors; Gαi: inhibitory subunit of G alpha proteins; Gαs: stimulatory subunit of G alpha proteins; ADCY: adenylate cyclase; PKA: cAMP-dependent protein kinase A; CREB: cAMP responsive element binding protein; NFKB: nuclear factor-kappa B; RTK: receptor tyrosine kinases; PI3K: phosphatidylinositol-4,5-bisphosphate 3-kinase; ERK: mitogen-activated protein kinase 1 and 3; AKT: v-akt murine thymoma viral oncogene homolog 1; GABA(A)R: inotropic GABA receptors; PR: nuclear progesterone receptor; EGR2: early growth response 2 transcription factor; PROG: progesterone, DHP: dihydroprogesterone; THP: allopregnanolone, positive modulator of GABA(A)R receptors. PROG, DHP and THP are neurosteroids produced by Schwann cells. cAMP: cyclic AMP; PDGF: platelet-derived growth factor; IGF1: insulin-like growth factor 1; NRG1: neuregulin 1; “silencer”: putative cAMP-dependent regulatory region in the promoter of *PMP22* gene.

(B) Cytoprotective and neuromodulator actions of PXT3003 drug combination in peripheral neurons. The reciprocal interactions of neuronal and Schwann cells assure correct processing of sensory and locomotor information in the peripheral nervous system (PNS) [14,

15]. We supposed that primarily functional abnormalities, induced by PMP22 overexpression in CMT1A Schwann cells, provoke a cascade of pathophysiological alterations in neurons [16]. Therefore, as an additional selection criterion for clinical development, we evaluated the potential capacity of candidate drugs for attenuating these secondary destructive effects in neuronal cells.

Schwann cells regulate the level of several neurotransmitters (GABA, ATP and glutamate) and inflammatory proteins in PNS [17–24]. Glutamate and ATP play an important role as excitatory and cytotoxic neurotransmitters under pathological conditions and are implicated in perturbed nociceptive signalling associated with inflammatory and neuropathic pain [25–33]. Dysfunction of CMT1A Schwann cells can not only disturb myelination process, but probably also affect levels of these neuromodulator substances, which can significantly compromise functional performance of neuronal signalling. Several publications demonstrated the excessive activation P2RX7 receptors, which modulate processing and release of CNTF and IL1B, in CMT1A Schwann cells [19, 34]. We hypothesized that increased neuronal excitability of sensory and motor neurons is responsible for development of at least some of pathological manifestations of CMT1A and represents an important functional target for therapeutic intervention in Charcot-Marie-Tooth disease.

Both $GABA_B$ and opioid receptors are powerful modulators of neuronal excitability and painful sensation [35–38]. $GABA_B$ receptors agonized by baclofen are able to inhibit P2X3 receptor-mediated neuronal excitability of nociceptive neurons, and attenuate NMDA-activated current in the primary sensory neurons [39, 40]. Basic molecular mechanism underlying antinociceptive effects of $GABA_B$ and opioid receptors can be mediated by the coupling of both receptors to activation G protein-gated inwardly rectifying K+ (GIRK) channels and inhibition of voltage-gated Ca^{2+} channels, though these metabotropic receptors are able also to modulate activity of TRPV1, voltage-gated sodium, ASICs and NMDA

receptors [38, 41–45]. Although not considered a significant symptom, pain is frequently complained by CMT1A patients [46, 47]. We expect that PXT3003 combination could attenuate sensory impairments accompanied development of Charcot-Marie-Tooth disease.

$GABA_B$ and opioid receptors are not only potent modulators of neuronal excitability, but are also able to activate several cytoprotective signalling pathways in different experimental settings (some of the established signalling modules, implicated in the anti-apoptotic effect of $GABA_B$ and opioid receptors, are shown) [35, 45, 48–51]. For instance, both $GABA_B$ and opioid receptors can protect neuronal cells from apoptosis by transactivation of IGF-1R receptor [52–55]. Importantly, neurotropic insulin-like growth factor-1 (IGF-1) not only protects cell from various cytotoxic insults, but also promotes axonal growth from dorsal root ganglion (DRG) neurons [56].

Finally, muscarinic receptors, that might mediate therapeutic effect of sorbitol, are also recognized as important functional receptors in PNS, modulate neuronal activity of primary sensory neurons, are implicated in nociceptive sensation and could provide substantial neuroprotection from broad spectrum of cytotoxic factors [49, 57–59].

We suppose that PXT3003 combination could preserve functional integrity of neuronal cells in CMT patients by attenuating excessive excitability of peripheral neurons, normalize propagation of neuronal impulses and reduce axonal loss and functional perturbations at neuro-muscular junctions.

IGFR1: insulin-like growth factor 1 receptor; PKC: protein kinase C; SRC: v-src avian sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog; AMPK: AMP-activated kinase; FAK: Focal adhesion kinase; BCL2: B-cell CLL/lymphoma 2; CACNAs: calcium channels, voltage-dependent; GRINs: ionotropic NMDA glutamate receptors; VGSC: sodium channels, voltage-gated; TRPV1: transient receptor potential cation channel, subfamily V, member 1;

ASICs: acid-sensing (proton-gated) ion channels; P2X3: purinergic receptor P2X, ligand-gated ion channel, 3; GIRKs: G protein-coupled inwardly-rectifying potassium channels.

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